

O-120.**Integrated transcriptomic and functional immunological approach for assessing the invasiveness of bivalve alien species**A. Romero, R. Aranguren, R. Moreira, B. Novoa[#], A. Figueras.

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Abstract

Biological invasions started when humans moved species beyond their normal geographic limits. Bivalves are the most notoriously invasive species in subtidal aquatic environments. Next-generation sequencing technologies are applied to understand the molecular mechanisms involved in the invasion. The ecological immunology focuses on the role of immunity in invasion, and its magnitude could help to predict the invasiveness of alien species. A remarkable case of invasion has been reported in the Ría de Vigo (Spain) by the black pygmy mussel *Xenostrobus securis*. In Galicia, the Mediterranean mussel *Mytilus galloprovincialis* is the predominant cultured bivalve species. Can we predict the invasiveness of alien bivalve species by analyzing their immune response? Can *X. securis* represent a risk for the autochthonous mussel? We evaluated the suitability of the immune-related hypotheses in our model by using an integrated transcriptomic and functional immunological approach. Our analysis suggests lower immune capabilities in *X. securis* compared to *M. galloprovincialis*, probably due to the relocation of energetic resources from the immune response to vital physiological processes to cope with salinity stress. This multidisciplinary approach will help us understand how the immune response can be influenced by the adaptive process and how this immune response can influence the invasion process.

Keywords: Ecological immunity, invasive species, immune response, mussel, transcriptome.

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O-121.**Transcriptomic analysis of clam extra pallial fluids reveals immunity and cytoskeleton alterations in the first week of brown ring disease development**Alexandra Rahmani^{1, #}, Erwan Corre², Gaëlle Richard¹, Adeline Bidault¹, Louisi Oliveira³, Fabiano Thompson³, Christine Paillard^{1, †}, Vianney Pichereau¹.

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Abstract

The Brown Ring Disease is an infection caused by the bacterium *Vibrio tapetis* on the Manila clam *Ruditapes philippinarum*. The process of infection, in the extrapallial fluids of clams, involves alteration of immune functions, in particular on hemocytes which are the cells responsible of phagocytosis. Disorganization of the actin-cytoskeleton in infected clams is a part of what leads to this alteration. This study is the first transcriptomic approach based on collection of extrapallial fluids on living animals experimentally infected by *V. tapetis*. We performed differential expression analysis of transcripts from healthy against infected clams by *V. tapetis*. We highlighted, in infected clams, a downregulation of transcripts implied in immune functions that might suggest an important role of deregulation of lysosomal activity and complement- and lectin-dependent PRR pathways during infection. We have also shown a deregulation of transcripts coding for proteins involved in actin cytoskeleton regulation

such as an overexpression of b12-Thymosin (which are actin sequestration proteins) or a downregulation of proteins that closely interact with capping proteins such as Coactosin that counteract action of capping proteins or Profilin. According to our results we made the hypothesis that *V. tapetis* might be able to force hemocytes to stay in a "resting state" to inhibit its phagocytic power.

Keywords: Brown Ring Disease, Hemocytes, Actin cytoskeleton, b-thymosin, Coactosin, Resting cells

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O-122.***Vibrio aestuarianus* virulent traits : Insights from in vitro interaction with oyster hemocytes**A. Mesnil¹, D. Tourbiez¹, C. Garcia¹, C. Lambert², M.A. Travers^{1, #}.

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Abstract

Oyster mortalities associated with pathogenic vibrios are a major concern for the sustainability of oyster farming. Since 2001 and notably since 2011, one bacteria has been regularly detected in France and recently in Ireland: *Vibrio aestuarianus*. Its implication in oyster mortalities has been validated through experimental pathology and field survey in France⁴. Moreover, its pathogenesis has been recently elucidated: after a quick colonisation of hemolymph, *V. aestuarianus* virulent strain proliferates and colonizes connective tissues in gills, digestive gland and mantle. However, shared virulence properties, specific to virulent strains, allowing bacterial proliferation in presence of hemocytes are still poorly described. This study aimed to determine *V. Aestuarianus* virulence strategies, exploring in vitro interactions between bacteria and hemocytes. Adult oysters hemocytes were exposed to virulent or non-virulent strains. Firstly, to identify common phenotypic trait we compared hemocyte response face to 8 virulent and non-virulent strains. And secondly, in a more targeted approach, a virulent strain (12/016) and its non-virulent mutant (12/016dVars, previously described) were also compared. Kinetics of hemocytes responses (phagocytosis, mortality and ROS production) were measured by flow cytometry. Moreover, bacterial proliferation in hemolymph was also estimated for virulent and non-virulent strains and will be presented.

Keywords: *Vibrio aestuarianus*, oysters, hemocytes, in vitro, phagocytosis

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O-123.**Immune response of common carp to presporogonic development of myxozoan *Sphaerospora molnari***T. Korytár^{1,2, #}, G.F. Wiegertjes³, E. Zusková², A. Tomanová⁴, M. Lisnerová^{1,4}, S. Patra¹, V. Sieranski^{4,5}, R. Šíma¹, A. Born-Torrijos¹, A.S. Wentzel⁶, S. Blasco-Monleon¹, C. Yanes-Roca², T. Polícar², A.S. Holzer¹.

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Abstract

Sphaerospora molnari is a myxozoan parasite causing skin and gill sphaerosporosis in common carp (*Cyprinus carpio*) in Central Europe. Prior to spore formation, multicellular proliferative stages of *S. molnari* circulate for several weeks in the vascular system of its host despite the array of humoral and cellular immune effector mechanisms. Using our laboratory infection model, we aimed to elucidate the kinetics of presporogonic development of *S. molnari*, while simultaneously analyze the immune responses over a period of 63 days. The obtained results identified two peaks of acute parasitemia on day 28 and 42 respectively. Unexpectedly, the highest parasite load was detected in the liver, a previously unknown localization of *S. molnari*. In response to the infection, the immune system induced dynamic changes in the expression of pro- and anti-inflammatory cytokines, with a predominant role of IL-10 reaching up to 1456 fold increase compared to control fish. The haematological analysis revealed a steady increase in the number of lymphocytes from day 28 onwards, correlating with the growing number of parasites, and only marginal changes in other populations. Additionally, our data revealed a strong increase in the expression of IgM transcripts and increased number of IgM⁺ B lymphocytes, which produce specific antibodies recognizing *S. molnari* antigens in western blot. Strikingly, although the sera of infected fish exhibit potent opsonizing capacity *in vitro*, *S. molnari* isolated from the blood of infected fish are not labelled with carp IgM. These findings indicate the presence of so far unknown evasion strategy and questions the importance of *S. molnari*-specific antibodies in parasite elimination. To our knowledge, this is the first study analyzing the early myxozoan development and immune modulation mechanisms along with innate and adaptive immune responses of the fish host in a controlled laboratory system, adding important information on host-parasite interaction of early metazoans (Cnidaria) with basic vertebrate immune systems.

Keywords: *Sphaerospora molnari*, Antibodies, Cytokines, B lymphocytes, IL-10

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O-124.

From gross morphology to gill transcriptome in farmed Atlantic salmon (*Salmo salar*): Lessons from multi-site sampling

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Abstract

The gill is a multifunctional organ involved in many physiological processes such as gas exchange, osmotic and ionic regulation, acid-base balance and excretion of nitrogenous waste. Due to its interface with the environment, the gill plays a key role as primary mucosal defence tissue

against pathogens and is equipped with the gill-associated lymphoid tissue (GIALT). In recent years, the prevalence of gill damage and gill diseases has increased significantly, leading to the substantial losses in Atlantic salmon aquaculture worldwide. Both the transition from healthy to unhealthy gill phenotypes as well the progression of various gill pathologies such as proliferative gill disease (PGD), amoebic gill disease (AGD) and complex gill disease (CGD) are commonly characterised by inflammation and epithelial cell hyperplasia. Routine monitoring for PGD relies on gross (macroscopic) evaluation of gill health, coupled with histological examination of gill sections. To explore underlying molecular events that are associated with progression of PGD, we examined Atlantic salmon from geographically diverse aquaculture sites in Scotland. Total RNA was extracted from 43 fish presenting low or medium gill PGD scores and analysed by whole transcriptome analysis using RNA-seq to determine the molecular signature of the advanced PGD. For each fish, 20M reads were generated and mapped to the Atlantic salmon genome. Importantly, we showed that the sampling site had greater effect on the gill transcriptome than the actual PGD score, providing support for a complex and multifactorial aetiology of PGD, with minimal common molecular responses between different sites. Similar pattern was found for histology, agreeing with the outcome of the RNA-seq analysis. In general, both RNA-seq and histology data clustered together based on the origin of samples, suggesting that the PGD scores may inform about the overall progression of gill damage, but not about the underlying pathology.

Keywords: Proliferative gill disease, gill histopathology, gill transcriptome, PGD scores, RNA-seq

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O-125.

A proteomic study of resistance to brown ring disease in the manila clam, *Ruditapes philippinarum*

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Abstract

Mollusks represent over a fifth of the global aquaculture market, accounting for USD 29.2 billion in 2016. Infectious disease is one of the main limiting factors to the development of mollusk aquaculture, and the difficulties inherent to combating pathogens through antibiotic therapies or disinfection have led to extensive research on host defense mechanisms and host-pathogen relationships. It has become increasingly clear that innate immunity and genetic variability are key factors underlying disease resistance, and that genetic selection for resistance is essential for effective disease control. The Manila clam, *Ruditapes philippinarum*, is a main cultured bivalve species of economic interest produced on a global scale. While the species originates from Japan, it was introduced to European coasts for aquaculture in the early 1970s. In 1987, mass mortalities in clam landings on the Atlantic coast of France led to the identification of Brown Ring Disease (BRD) and of its etiological agent, *Vibrio tapetis*. BRD is characterized by bacterial colonization of the host's extra-pallial compartment, provoking abnormal conchiolin deposits along the inner surface of the shell. While some clams are capable of effectively combating the pathogen, others succumb to a chronic infection characterized by thick conchiolin deposits, low condition index, and death. Although a significant body of research has allowed us to gain a better understanding the